51. STABILIZATION OF PYRIDOSTIGMINE AS PREVENTIVE ANTIDOTE

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INTRODUCTION

Pyridostigmine (3-hydroxy-1-methylpyridinium bromide dimethylcarbamate) facilitates the transmission of impulses across the myoneural junction by inhibiting the destruction of acetylcholine by cholinesterase. In current therapy pyridostigmine tablets are useful in the treatment of myasthenia gravis being put on the market by Roche with the name Mestinon). In the form of manganese bromide salt, Arzneimittelwerk Dresden produced pyridostigmine with the name Kalymin 60. As a preventive antidote, pyridostigmine was produced by DuPhar.

Design of oral antidotes based on pyridostigmine meet some scientific and logistic real challenges. The logistics challenge is common to almost all antidotes – scarce utilization in therapy, high cost and as a consequence, low interest it concerns its developing by the pharmaceutical companies.

Another problem is not associated to all antidotes but is clearly the common disadvantage of all decorporators and to all antidotes utilizable in chemical intoxication with organophosphorus compounds. Following their ionic character these compounds have a poor absorption, low permeability inside biological cell and a rapid elimination, in other words a very unfavorable pharmacokinetics.

But pyridostigmine does not have only "common" problems of antidotes. A supplementary, "distinguished" characteristic is the very high hygroscopicity of pyridostigmine, with associated problems it concerns technological process and stability in "field of battle" conditions. Our research program was oriented in the following directions:

- choice of excipients and working parameters in order to assure reproducible technology and stable antidotes, elaboration of an in vitro release test discriminatory it concerns the release profiles; study of the interfacial transfer as an in vitro model for the trans-membrane transfer of pyridostigmine paired with organic anions
- development of a bioanalytical method for separation of pyridostigmine from plasma and chromatographic determination study of the pharmacokinetics in healthy volunteers and correlation of the results with in vitro data .

This paper presents *mainly the pharmaceutical technology* aspects and their influence on the biopharmaceutical properties of resulted tablets.

METHODS

Pharmaceutical formulas for tableting

Different formulas were compared, which relate to tabletting technology properties:

formula I : pyridostigmine 11.11%, bentonite 88.89%;
formula II : pyridostigmine 5.55%, bentonite 44.45%, amidon 50%;
formula III : pyridostigmine 5.41%, bentonite 43.36%, amidon 48.78% and magnesium stearate 2.44%.

In vitro dissolution tests

apparatus 2 (paddle),
method : USP XXIV,
volume : 900 ml,

agitation : 50 U/min,
sample time : 15, 30, 45 and 60 minutes,
medium : water, pH = 6; HCl 0.1N, pH = 2; phosphate buffer, pH = 8
tolerances : not less than 80% Q dissolved in 60 minutes.

Extraction of pyridostigmine from plasma

Liquid/liquid (L/L) extraction[1] -1 mL plasma sample, mixed with 0.2ml phosphate buffer and 0.5ml picric acid 66mM, was extracted with dichlormethane (2 times 4ml). The organic extract was treated with 0.2ml tetrabutylammonium iodide 1mM. The aqueous layer was analyzed by HPLC, on a Zorbax-CN column, equipped with a pre-column of the same material. Isocratic elution at 40°C temperature has been preferred, using a flow rate of 1.2ml/min. Mobile phase consists in a mixture acetonitrile-triethylamine-acetic acid. Injected sample volumes were 200µL and chromatograms were monitored at 270 ± 2 nm.
It was tried solid phase extraction (SPE) with different type of stationary phase, cyane and Oasis-HLB - [poly(divinylbenzene-co-N-vinylpyrolidone)].

Evaluation of bioavailability of pyridostigmine

4 male young volunteers were enrolled in the pharmacokinetic study. All volunteers were healthy as assessed by physical examination and laboratory tests. After an overnight fast of ten hours, the volunteers received 2 tablets of 18 mg of pyridostigmine. Venous blood samples were collected through a catheter at 0,0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4 and 6 hours after drug administration. Plasma samples were prepared by L/L extraction and analyzed by HPLC.

RESULTS

Tableting technology and storage in "field conditions"

As it was presented the main difficulty in achieving pharmaceutical forms with pyridostigmine arise from its hygroscopicity.

The first trials started from the formulas containing same excipients as Mestinon (lactose, magnesium trisilicate, calcium phosphate and magnesium stearate) or as Kalimyn (lactose, amidon, talc, magnesium stearate and polividon 25). Working in "open air" conditions it was not possible to avoid the absorption of water from atmosphere and the resulted mixture of powders was not directly compressible.

Lyophilization of pyridostigmine allowed direct compression in an interval of some hours but the resulted tablets were not stable in time.

A powder of only bentonite and pyridostigmine was stable and direct compressible for 10 years. Since the adsorption of pyridostigmine on bentonite was proved by in vitro dissolution test, it was tried to replace partially the bentonite by amidon. Replacing partially bentonite with amidon did not change the stability of tablets if the proportion of amidon remained less or equal with bentonite.

In vitro dissolution tests

In vitro dissolution test indicated a strong absorption on bentonite, whatever the dissolution medium (water, acidic or basic media), the quantity released in one hour being less than 40 % of the total pyridostigmine.

As it can be seen from figure 1, in acidic and neutral media the curves reach very quickly (practically after half an hour) a plateau. It was considered that is not necessary to follow the dissolution for longer time interval than that indicated by USP 24 (one hour).
At pH=8, which is approximately the intestinal pH, the dissolution is somewhat better, a slow further increase of the released amount could appear after first hour. Consequently, we

consider that replacing of deaereted water with phosphate buffer would result in a more discriminatory dissolution test for pyridostigmine tablets with bentonite matrix. See Figure 1.

Extraction of pyridostigmine from plasma

Since pyridostigmine reachs not enough high plasma levels to allow a direct injection of samples in the chromatographic column, the extraction and the enrichment of the drug content of samples appeared as unavoidable steps of analytical assay procedures in the pharmacokinetic study. Consequently it was tried both SPE and L/L extraction as methods for separation of drug from plasma matrix.

For a good reproducibility of SPE it is essential to have a good wettability of the sorbent. In the last years, it was developed new type of macroporous copolymer exibiting both hydrophilic and lipophilic retention characteristics[2]. This type of sorbent is theoretically more efficient in retaining hydrophilic pyridostigmine molecule than usual silica sorbents. Our expectetion were not fulfilled, the extraction yield from aqueous sample being 10% and only 5% in case of plasma samples.

Since best results in chromatographic separation of pyridostigmine were obtained in the case of CN columns, we thought that it deserves to use for SPE same type of sorbent. The extraction yield was really 7 fold greater that in the case of OASIS. A possible explanation for this result could be connected with the bound of quaternary ammonium in the area of the high electron density of the CN group. See Figure 2.

Since use of good laboratory practice implies a single use of the separation cartridge, SPE methods remain enough expensive to be commonly used in pharmacokinetic studies. Although less selective, the L/L extraction presents the advantage of a greater flexibility following the adjustment of parameters which control the process (pH, nature and volume of the extraction solvent, etc). Last but not least the L/L extraction is by far less expensive than SPE. Consequently, in pharmacokinetic study for sample preparation it was tried also L/L extraction.

Since pyridostigmine is practically insoluble in all organic solvents is not possible to obtain an acceptable recovery from plasma whatever the combination of extracting solvents. A solution of the problem is the "derivatization" of pyridostigmine by association with a negative ion to form a dissociable ion pair. Utilization of picrate as counter ion, allowed extraction in methylene-chloride of approximately 40% of pyridostigmine from spiked plasma samples.

Such type of extraction by intermediate of ion pair could be considered as a model for a type of "in vivo" facilitated transport across lipid membranes.

Evaluation of bioavailability of pyridostigmine from bentonite matrix

As we presented, "in vitro" experiments seem to indicate a retention of pyridostigmine from bentonite. Since there are many examples of drugs, which are bioequivalent though their in vitro dissolution profiles are enough different, we considered that final, definitive conclusion about the bioavailability of pyridostigmine would remain the in vivo experiment.

The plasma levels of pyridostigmine at healthy volunteers are presented in figure 3. It can be observed that the intersubject variability is high, though high C_{max} at volunteer II makes the other three volunteers to look very closely.

Pharmacokinetic study See Figure 3.

The same conclusion is supported by the examination of the calculated pharmacokinetic parameters. On other hand, if the variability of C_{max} and T_{max} is a normal phenomenon in conditions of "poor" absorption, the differences in $T_{1/2}$ are rather unexpected. See TABLE 1.

Whatever the explanations of these anomalies it is important for our study to note that plasma levels are of the same magnitude with that obtained in other studies [3,7], supporting the idea of a modification of the bentonite matrix structure at the intestinal pH, allowing enough absorption for obtaining measurable plasma levels for some hours.

CONCLUSIONS

I. Bentonite as excipient assures:

- good and stable technology conditions for tableting pyridostigmine even in "open air" conditions,
- stability of pyridostigmine tablets even in worse climatic conditions for more than 10 years

In vitro evaluation of tablets proves a strong adsorption of the active substance on bentonite. In vivo experiment on healthy volunteers indicates that the absorption is probably reduced but significant plasma levels are attained for some hours.

Pyridostigmine tablets with bentonite matrix are easy to obtain and stable even in "field" conditions. The exact dose of active substance to be used can be established only after in vivo pharmacologic experiments.

REFERENCES

1. Terry Sarah, Zvi Teitelbaum: *Determination of pyridostigmine in human plasma by high-performance liquid chromatography*, Dec 1991, J Liq Chromatogr, 14(20), 3745-54
2. Waters - OASIS™ HLB extraction cartridges, 1996
3. Aquilonius SM, Eckernas SA, Hartvig P, Lindstrom B, Osteman P: *Pharmacokinetics and oral bioavailability of pyridostigmine in man*, Eur J Clin Pharmacol, nov 1980, 18(5), 423-8
4. Marino MT, Schuster BG, Bruueckner RP, Lin E, Kaminskis A, Lasseter KC: *Population pharmacokinetics and pharmacodynamics of pyridostigmine bromide for prophylaxis against nerve agent in human*, J Clin Pharmacol, 1998 mar, 38(3), 227-35
5. Breyer-Pfaff U, Mayer U, Hartvig P, Lindstrom B, Osterman PO: *Pyridostigmine kinetics in healthy subjects and patients with myastenia gravis*, Clin Pharmacol Ther 37, 495, 1985
6. Calven TN, Chan K: *Kinetics of intravenous pyridostigmine in man*, Br. J. Clin. Poharmacol, 1981, 11(4), 406-8
7. Cohan SL, Pohlmann JL, Mikszewski J, O'Doherty DS: *The pharmacokinetics of pyridostigmine*, Neurology, 1976, 26(6PT 1), 536-9

FIGURES AND TABLE

Figure 1. Pyridostigmine release profile in different dissolution media

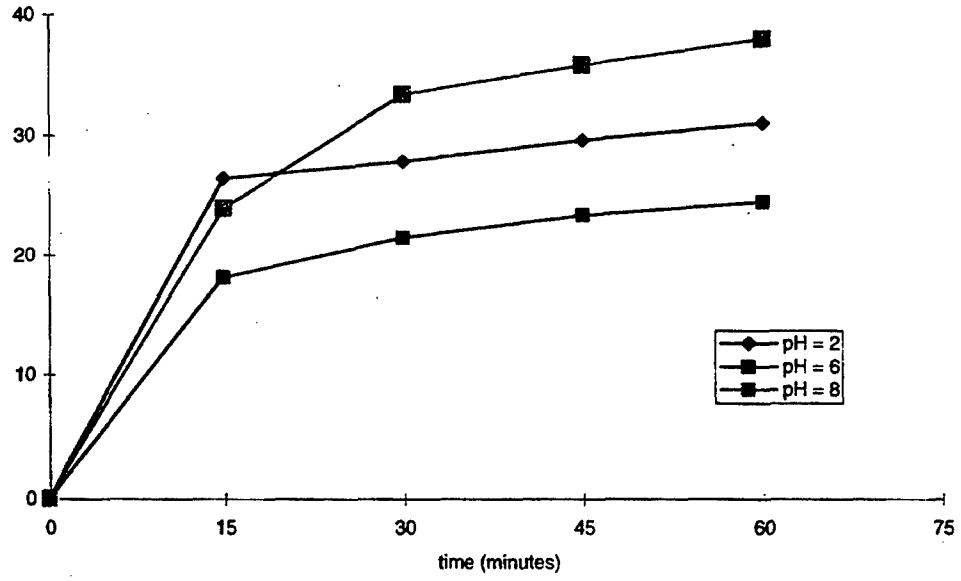

Figure 2. Pyridostigmine SPE on CN and OASIS columns

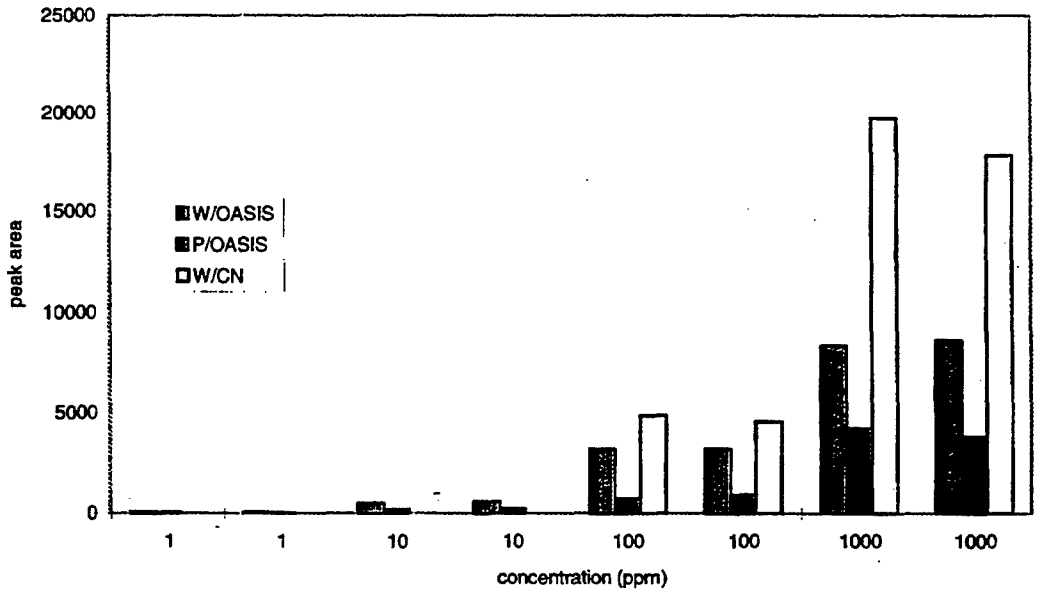

Figure 3. Plasma levels of pyridostigmine after oral administration to 4 healthy volunteers

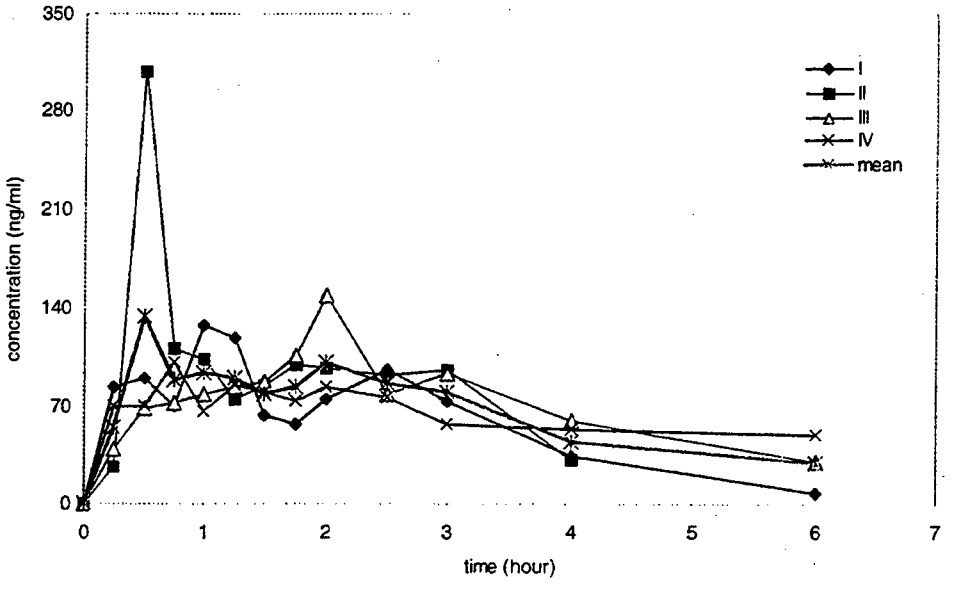

Table 1. Main pharmacokinetics parameters of pyridostigmine administered to 4 healthy volunteers

VOLUNTEER COD	CMAX (ppb)	TMAX (h)	T1/2 (h)	AUD (ppb* h)	AUC (ppb* h)	MRT	ClT	Vd
I	127	1	0.93	341.01	350.92	2.31	2370	192
II	309	0.5	0.83	410.4	419.2	2.11	1990	143
III	150	2	2.98	567.92	909.54	5.85	916	236
IV	100	0.75	2.86	375.05	576.55	5.33	1450	358
mean	134	0.5	2.85	433.06	619.24	4.91	1350	332